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DATE: Thursday, May 27, 2004 Printable Copy Create Case

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DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ			
L35	l32 same l22	31	L35
L34	l32 same l1	2	L34
L33	L32 same l4	8	L33
L32	l29 with l5	406	L32
L31	L29 with (l5 or l4)	1426	L31
L30	L29 with l1	3	L30
L29	citraconic anhydride or N-Hydroxysuccinimide acetate or cca or NHS-acetate	19785	L29
L28	6379966	8	L28
L27	l25 same l1	6	L27
L26	L25 same l18	4	L26
L25	stable colloid	403	L25
L24	L21 same l23	80	L24
L23	L22 or l1	564835	L23
L22	stable colloid or neutral or anionic	545347	L22

L21	L20 with l4	877	L21
L20	l18 with l5	135774	L20
L19	L18 same l17	3	L19
L18	PEG or poly(alkylene oxide) or polyethylene or citraconic anhydride or N-Hydroxysuccinimide acetate or cca or NHS-acetate	735521	L18
L17	L16 same l5	54	L17
L16	l1 with l4	114	L16
L15	L14 same l4	1	L15
L14	L13 with l5	11	L14
L13	L12 with l1	172	L13
L12	L11 or l10	2788123	L12
L11	modifies	319669	L11
L10	modif$	2667001	L10
L9	l7 and l4	2	L9
L8	L7 same l4	1	L8
L7	l5 with l3	20	L7
L6	L5 with l4 with l3	1	L6
L5	amphiphile or lipid or liposome or polymer	1829042	L5
L4	dna or polynucleotide or gene or nucleic or plasmid	371096	L4
L3	L2 with l1	1224	L3
L2	reduc? or revers?	3188590	L2
L1	surface potential or zeta potential	22723	L1

END OF SEARCH HISTORY

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L24: Entry 30 of 80 File: PGPB Jan 23, 2003

DOCUMENT-IDENTIFIER: US 20030017972 A1
TITLE: Complexing agents for compositions containing inclusion complexes

Detail Description Paragraph:
[0316] Polyplexes (polymer to DNA charge ratio of 3+/-) modified with Tf-PEG-AD (or Tf-(PEG-AD).sub.2) and PEG-AD (or PEG-Glu-Glu-AD) can be formulated as follows. Equal volumes of all components are used. Tf-PEG-AD(or Tf-(PEG-AD).sub.2) in water is added to a solution of 12 in water. To this mixed solution is added an aliquot of PEG-AD (or PEG-Glu-Glu-AD). The ternary mixture of polymers is then added to DNA solution. The solutions are mixed gently by pipeting and particle size, zeta potential, and salt stability determined as described previously. The zeta potential of the particles can be tuned by varying the relative ratios of Tf-PEG-AD (or Tf-(PEG-AD).sub.2) vs. PEG-AD (or PEG-Glu-Glu-AD). Some examples of zeta potential variation and particle size as a function of particle modification is shown in FIGS. 26, 27, and 28.

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L24: Entry 34 of 80 File: PGPB Dec 5, 2002

DOCUMENT-IDENTIFIER: US 20020182249 A1
TITLE: Preparation of stable formulations of lipid-nucleic acid complexes for efficient in vivo delivery

Detail Description Paragraph:
[0032] The term "lipid:nucleic acid complex" refers to the product made by mixing amphiphilic cationic lipids or liposomes with a nucleic acid. The term "CLDC," which stands for "cationic lipid:DNA complex" as used herein is not limited to DNA and is a convenient abbreviation for lipid:nucleic acid complex. The lipid:nucleic acid complex can also include a helper lipid. The helper lipid is often a neutral lipid such as DOPE or cholesterol with cholesterol being most preferred. The lipid:nucleic acid complex may also contain other compounds such as a polycation that are in contact with the nucleic acid of the complex, producing condensed nucleic acid, and hydrophilic polymers such as PEG and derivatized PEG.

L24: Entry 68 of 80 File: USPT Jun 1, 1999

DOCUMENT-IDENTIFIER: US 5908777 A
TITLE: Lipidic vector for nucleic acid delivery

Detailed Description Text (21):
In accordance with the present invention, the aforementioned 20-mer peptide, with its three negatively charged glutamic acid residues, was added to a positively charged DNA/polylysine complex at a DNA/polylysine/20-mer peptide ratio of 1:0.75:0.4 (wt:wt:wt). The resultant complex then was encapsulated into anionic liposomes composed of DOPE/CHEMS/folate-PEG-PE (6:4:0.01) at a lipid/DNA ratio of 12:1 (wt:wt). These DNA-containing liposomes were highly effective in transfecting receptor-bearing KB cells, and remained effective in the presence of 10% fetal bovine serum. By contrast, liposomes lacking the 20-mer peptide lost transfection effectiveness in the presence of serum.

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L33: Entry 1 of 8 File: PGPB Feb 6, 2003

DOCUMENT-IDENTIFIER: US 20030026841 A1
TITLE: Compositions and methods for drug delivery using pH sensitive molecules

CLAIMS:

6. The process of claim 1 wherin the polyampholyte comprises one or more polyanions selected from the group consisting of to poly-L-aspartic acid, poly-D-aspartic acid, poly-L,D-aspartic acid, polyacrylic acid, poly-L-glutamic acid, poly-D-glutamic acid, poly-L,D-glutamic acid, succinylated poly-L-lysine, succinylated poly-D-lysine, succinylated poly-L,D-lysine, succinylated polyethylenimine, succinylated polyallylamine, succinylated poly-L-ornithine, succinylated poly-D-omithine, succinylated poly-L,D-omithine, succinylated polyvinylamine, polymethacrylic acid, dextran sulfate, heparin, hyaluronic acid, DNA, RNA, natural anionic proteins, synthetic anionic proteins, synthetic anionic peptides, and synthetic polymers contining monomers in which an amine has been reacted with a substructure of citraconic anhydride and/or substructure of maleic anhydride.

13. The process of claim 9 wherin the polyampholyte comprises one or more polyanions selected from the group consisting of poly-L-aspartic acid, poly-D-aspartic acid, poly-L,D-aspartic acid, polyacrylic acid, poly-L-glutamic acid, poly-D-glutamic acid, poly-L,D-glutamic acid, succinylated poly-L-lysine, succinylated poly-D-lysine, succinylated poly-L,D-lysine, succinylated polyethylenimine, succinylated polyallylamine, succinylated poly-L-ornithine, succinylated poly-D-ornithine, succinylated poly-L,D-omithine, succinylated polyvinylamine, polymethacrylic acid, dextran sulfate, heparin, hyaluronic acid, DNA, RNA, natural anionic proteins, synthetic anionic proteins, synthetic anionic peptides, and synthetic polymers contining monomers in which an amine has been reacted with a substructure of citraconic anhydride and/or substructure of maleic anhydride.

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      (FILE 'HOME' ENTERED AT 17:50:29 ON 27 MAY 2004)

      FILE 'MEDLINE, CANCERLIT, EMBASE, BIOTECHDS, BIOSIS, CAPLUS' ENTERED AT
      17:50:47 ON 27 MAY 2004
L1         31551 S ZETA POTENTIAL OR SURFACE POTENTIAL
L2       3671467 S DNA OR NUCLEIC OR POLYNUCLEOTIDE OR PLASMID
L3       8427618 S MODIF? OR REVERS? OR REDUC?
L4           344 S L3 AND L2 AND L1
L5       1140081 S CATIONIC LIPID OR AMPHIPHILE OR LIPOSOME OR POLYMER
L6           132 S L5 AND L4
L7        574932 S NEUTRAL OR ANIONIC OR STABLE COLLOID
L8         35171 S L7 AND L5
L9            40 S L7 AND L6
L10           17 DUP REM L9 (23 DUPLICATES REMOVED)
L11        10243 S CITRACONIC ANHYDRIDE OR N-HYDROXYSUCCINIMIDE ACETATE OR CCA O
L12            9 S L11 AND L1
L13            7 DUP REM L12 (2 DUPLICATES REMOVED)
L14           19 S L11 AND L5 AND L2
L15           15 DUP REM L14 (4 DUPLICATES REMOVED)

=>
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L10 ANSWER 13 OF 17 MEDLINE on STN DUPLICATE 9
AN 2001052222 MEDLINE
DN PubMed ID: 11018552
TI Characteristics and biodistribution of cationic liposomes and their **DNA** complexes.
AU Ishiwata H; Suzuki N; Ando S; Kikuchi H; Kitagawa T
CS Pharmaceutical Formulation Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Tokyo R&D Center, 16-13 Kita-Kasai 1-Chome, Edogawa-ku, Tokyo 134-8630, Japan.. ishiw9fq@daiichipharm.co.jp
SO Journal of controlled release : official journal of the Controlled Release Society, (2000 Oct 3) 69 (1) 139-48.
Journal code: 8607908. ISSN: 0168-3659.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200012
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001213
AB We have developed some novel **liposome** formulations for gene transfection. The formulations consisting of O,O'-ditetradecanoyl-N-(alpha-trimethyl ammonio acetyl) diethanolamine chloride (DC-6-14) as a **cationic lipid**, phospholipid and cholesterol showed effective gene transfection activity in cultured cells with serum and in vivo, i.e., intraperitoneal injection in mice. In this report, the physicochemical characteristics and biodistribution of the liposomes containing DC-6-14 (DC-6-14 liposomes) as a drug (gene) carrier for gene therapy were investigated in vitro and in vivo. DC-6-14 **liposome -DNA** complexes were usually thought to have positive surface charge. However, depending on the ratio of **DNA** to liposomes, **zeta-potential** of the complexes became negative. The diameter of the complexes also depended on the **DNA-liposome** ratio, and showed a maximum when their **surface potential** was **neutral**. When biodistribution of the complexes was determined after intravenous injection, positively charged complexes showed an immediate lung accumulation. On the other hand, negatively charged complexes did not show lung accumulation. These results have suggested that biodistribution of the **DNA-liposome** complexes, prepared with DC-6-14 liposomes, depends on their surface charge. Therefore, some surface **modification** of DC-6-14 liposomes may improve the biodistribution and hence the targetability of their **DNA** complexes.

L10 ANSWER 17 OF 17 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 12

AN 1998091844 EMBASE

TI Modulation of cationic liposomal **DNA zeta potential** and **liposome**-protein interaction by amphiphilic poly(ethylene glycol).

AU Phillips N.C.; Heydari C.

CS N.C. Phillips, Faculty of Pharmacy, University of Montreal, CP 6128, Montreal, Que. H3C 3J7, Canada

SO Pharmaceutical Sciences, (1996) 2/2 (73-76).
Refs: 24
ISSN: 1356-6881 CODEN: PHSCFB

CY United Kingdom

DT Journal; Article

FS 029 Clinical Biochemistry

LA English

SL English

AB In an attempt to **reduce** the surface charge of cationic liposomes, and thereby increase their transfection efficiencies, the effect of the amphiphilic solvation enhancer dipalmitoylphosphatidylethano laminyl-poly(ethylene glycol) (DPPE-PEG) on the stability of cationic dioleoylphosphatidyletanolamine (DOPE) dioleoyltrimethylammonium propane (DOTAP) liposomes, their interaction with **DNA** and the aggregation of liposomal **DNA** complexes by **anionic** proteins has been evaluated by photon correlation spectroscopy and measurement of **liposome .zeta. potential**. DOPE-DOTAP liposomes were unstable, and exhibited significant aggregation after seven days storage at 4°C. DOPE-DOTAP liposomes containing DPPE-PEG (5 mol%) were more stable, but also showed some aggregation. DOPE-DOTAP liposomes had a **.zeta. potential** of +34 mV. This was significantly **reduced** to a value of +6 mV by the incorporation of DPPE-PEG. Both **liposome** formulations reacted with **DNA** at weight ratios of 1:1 to 15:1 within 1-5 min at pH 7.4 and 23°C. The **.zeta. potential** of DOPE-DOTAP liposomes was significantly **reduced** by genomic and **plasmid DNA**, in a dose-dependent manner, to give a **.zeta. potential** of +3 mV at a **liposome**-to-**DNA** ratio of 1:1. The **.zeta. potential** of DOPE-DOTAP-DPPE-PEG liposomes was further **reduced** by **DNA** to -9 mV at a **liposome**-to-**DNA** ratio of 1:1. Incubation of DOPE-DOTAP liposomal **plasmid DNA** (1:5 ratio) with the **anionic** proteins albumin or IgG, or with a buccopharyngeal wash resulted in a rapid and significant aggregation(0.18 μm to 1-2 μm) accompanied by significant **reductions** in **.zeta. potential**. In contrast, DOPE-DOTAP-DPPE-PEG liposomes showed only a slight increase in size that was not accompanied by a significant change in **.zeta. potential**. These results indicate that although DPPE-PEG masks the positive charge of DOTAP at the **liposome** surface and thus **reduces** electrostatic interaction with **anionic** proteins, it still enables efficient interaction of DOTAP with genomic and **plasmid DNA**.

L10 ANSWER 1 OF 17 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AN 2004-04580 BIOTECHDS
TI A liposomal complex which has a masking agent **reversibly** associated with the external surface, useful for the delivery of **nucleic** acids or drug products to target tissues whilst bypassing non-target tissues or organs;
liposome-mediated **DNA** transfer and expression in host cell for gene therapy
AU SMYTH TEMPLETON N
PA BAYLOR COLLEGE MEDICINE
PI US 2003180950 25 Sep 2003
AI US 2003-393101 20 Mar 2003
PRAI US 2003-393101 20 Mar 2003; US 2002-366764 22 Mar 2002
DT Patent
LA English
OS WPI: 2003-898829 [82]
AB DERWENT ABSTRACT:
NOVELTY - A liposomal complex for drug delivery, comprising a pharmaceutical surrounded and protected by a **cationic lipid** layer and a masking agent **reversibly** associated with an exterior surface of the lipid layer, where the masking agent inhibits first pass clearance of the complex by a lung tissue, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for (a) an **anionic** masking agent for **reversibly** interacting with an exterior surface of a cationic liposomal complex which has a molecular weight of about at most 5000 Da and which inhibits first pass clearance of the **liposome** by a lung tissue; (b) optimizing the delivery of targeted liposomal complexes, comprising selecting a **reversibly** interacting masking agent for a particular liposomal complex, titrating the liposomal complex with the masking agent to determine an amount of masking agent necessary to achieve a desired **zeta potential**, mixing the liposomal complex with the determined amount of masking agent to form a masked liposomal complex, and testing the complex for delivery to a target tissue; (c) selecting potential liposomal masking agents, comprising: (a) selecting a number of compounds that interact with an external lipid layer of a liposomal complex; (b) mixing a number of concentrations at most 18 mM of each of the selected compounds with a liposomal complex containing a label to form a masked labeled liposomal preparation; (c) incubating a predetermined number of cells from a mammalian cell line with each of the masked labeled liposomal preparations; (d) determining the preparations that decreased uptake of the label into the cell line; (e) performing in vivo assessments of a tissue uptake of the label when the preparations from (d) are systemically administered to an animal; and (f) selecting the compound used in the preparations that are taken up by the tissue.
BIOTECHNOLOGY - Preferred complex: The liposomal complex further comprises a targeting ligand associated with the exterior surface of the lipid layer. A dissociation constant of the masking agent with the lipid layer surface is greater than a dissociation constant of the targeting ligand and the lipid layer surface. The masking agent has a molecular weight of less than the targeting ligand. The pharmaceutical includes a **polynucleotide**, particularly a **plasmid**, or a peptide.
The complex preferably has an internal and an external lipid bilayer, with the pharmaceutical encapsulated between the internal and external bilayers. More preferably there are a pair of internal and a pair of external lipid bilayers. The **liposome** is preferably a bilamellar invaginated **liposome** comprising an extruded mixture of DOTAP and cholesterol. The masking agent has a molecular weight of at least about 5000 Da and the targeting ligand has a molecular weight of at least about 10000 Da, or alternatively the masking agent is at most 2000 daltons and the targeting ligand is 2000-15000 Da, or the masking agent is at most 500 Da and the targeting ligand is 500-5000 Da. The masking agent is a lipid, **anionic** or **neutral** lipophilic

compound, most preferably n-dodecyl-beta-D-maltopyranoside or a polyethylene glycol derivative with a molecular with of about 5000 Da. The masking agent is in a concentration of about 3-10 mM. The liposomal complex has a **zeta potential** of about 3-10 millivolts and a mean particle size of about 200-500 nm (claimed).

USE - The complex is useful for the delivery of **nucleic** acid or drug product to target tissues whilst bypassing non-target tissues or organs.

EXAMPLE - 6 week old Balb/c mice were injected in the tail veins with 200 microl DOTAP:Chol:**DNA** complexes. The **DNA** was a chloramphenicol acetyltransferase (CAT) reporter **plasmid** that would lead to expression of CAT in successfully transfected target cells. The masking agent used on the complexes was a 5000 MW polyethylene glycol **polymer** with 5 pendant carboxyl groups. The mice were sacrificed 24 hours post injection and the heart and lung harvested and frozen. Standard CAT ELISA was used to determine CAT expression. In both lung and heart the expression of CAT was significantly lower when the liposomal complexes were masked with the PEG **polymer** (see figure).(17 pages)

L13 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:696467 CAPLUS
DN 139:235406
TI Polynucleotide complex delivery
IN Monahan, Sean D.; Wolff, Jon A.; Hagstrom, James E.; Budker, Vladimir G.; Rozema, David B.; Slattum, Paul M.
PA USA
SO U.S. Pat. Appl. Publ., 25 pp., Cont.-in-part of U.S. Ser. No. 450,315.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 13

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2003166280	A1	20030904	US 2002-85378	20020227
	US 2001019723	A1	20010906	US 1999-450315	19991129
	US 6379966	B2	20020430		
	WO 2003040375	A1	20030515	WO 2002-US17556	20020530
	W: JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
PRAI	US 1999-450315	A2	19991129		
	US 1999-121730P	P	19990226		
	US 1999-146564P	P	19990730		
	US 2001-12804	A	20011106		

AB Disclosed is a complex for providing nucleic acid expression in a cell. A polynucleotide and a polymer are mixed together to form the complex wherein the **zeta potential** of the complex is not pos. Then the complex is delivered to the cell wherein the nucleic acid is expressed. E.g., 5,5'-dithiobis(2-nitrobenzoic acid)-tetraethylenepentamine copolymer was prepared and DNA complexes of this polymer were injection into mouse tail and plasmid DNA was release from the complex and was accessible for transcription.